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#### APPENDIX F: THERMAL CYCLER 9600 HEATER, CHILLER, TEMPERATURE UNIFORMITY, AND TEMPERATURE CALIBRATION VERIFICATION DIAGNOSTIC TESTS.

- 1. DNA THERMAL CYCLER 9600 HEATER TEST
  - 1.1 At the main menu, press the OPTIONS key to move the cursor to the UTIL.

Select Options 9600 RUN- CREATE - EDIT -<u>U</u>TIL

1.2 Press ENTER. The utilities menu appears:

Select Function
DIR - CONFIG - DIAG - DEL

1.3 Press the OPTION key to move the cursor to the DIAG, press enter. The following display appears:

Enter Diag Test # 1 Review History File

1.4 Run the Heater Test by pressing 2, then ENTER. The following display appears:

Heater Test Blk = xx.xGoing to  $35^{\circ}C...$ 

When the temperature stabilizes, full power is applied to all heaters. The display reads "Ramping...", then "Timing..." and the block temperature is monitored.

When the block reaches the setpoint, the following screen appears:

Heater Test Passed

This display will show "Passed" if the test was successful. If the test was not successful, the display will show "Failed". If this should occur, contact an authorized vendor.

- 1.5 Press STOP to return to the first diagnostics display.
- 1.6 Refer to Appendix I for the appropriate worksheet to record the results.
- 2. DNA THERMAL CYCLER 9600 CHILLER TEST
  - 2.1 At the main menu, press the OPTIONS key to move the cursor to the UTIL.

Select Options 9600 RUN- CREATE - EDIT -<u>U</u>TIL

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2.2 Press ENTER. The utilities menu appears:

Select Function
DIR - CONFIG - DIAG - DEL

2.3 Press the OPTION key to move the cursor to the DIAG, press enter. The following display appears:

Enter Diag Test # 1 Review History File

2.4 Run the Heater Test by pressing 3, then ENTER. The following display appears:

Chiller Test Blk = xx.xGoing to  $50^{\circ}$ C...

The system first waits for the coolant temperature to reach 10°C. If the unit cannot do this within 18 minutes, a failure message will be displayed.

When the temperature stabilizes, the system drives the sample block cold, the temperature is monitored for a specific amount of time, and the cooling rate is calculated.

When the block reaches the setpoint, the following screen appears:

Chiller Test Passed

This display will show "Passed" if the test was successful. If the test was not successful, the display will show "Failed". If this should occur, contact an authorized vendor.

- 2.5 Press STOP to return to the first diagnostics display.
- 2.6 Refer to Appendix I for the appropriate worksheet to record the results.

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DNA THERMAL CYCLER 9600 TEMPERATURE CALIBRATION VERIFICATION TEST

3.1 Verifying the Temperature Calibration

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Perform the procedure as described in the following steps. It is recommended that you perform this procedure immediately after turning the instrument on because the sample block heated cover is not hot at that time.

- 3.1.1 If the sample block heated cover is in the forward position, turn the knob completely counterclockwise, then slide the cover back.
- 3.1.2 Coat wells D1 and E1 with mineral oil using a cotton swab.
- 3.1.3 Place the probe tray on the sample block so that the probe tray notch faces the front of the instrument.
- 3.1.4 Place the probe assembly into wells D1 and E1 so that the dummy probe sits in D1. Carefully thread the probe wire through the notch in the probe tray.
- 3.1.5 Slide the heated cover forward, then turn the cover knob clockwise until the white mark on the knob is aligned with the white mark on the cover.
- 3.1.6 Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.
- 3.1.7 Turn on the GeneAmp PCR System 9600. The main menu appears:

Select Option 9600 <u>RUN-CREATE-EDIT-UTIL</u>

3.1.8 Press the OPTION key three times to move the cursor to UTIL, then press ENTER. The utilities menu appears:

Select function
DIR-CONFIG-DIAG-DEL

3.1.9 Press the OPTION key twice to move the cursor to DIAG, then press ENTER. The following display appears:

Enter Diag Test #1 Review History file

3.1.10 Run the Verify Calibration Diagnostic Test (Test #5) by pressing 5 then ENTER.

\* The temperature of the sample block and heated cover will go to 40°C, and the following display will appear:

Going to  $40^{\circ}$ C... Cvr= xxC Blk = xx.xC

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This display shows the current temperature of the block cover (Cvr= xxC) and sample block (Blk = xx.xC).

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\* When the temperature of the block cover is within ten degrees of the sample block temperature, the following display appears:

Wait 3 minutes...
Time=MM:SS Blk=40.0C

This display shows the current sample block temperature ("Blk=40.0C") and a clock, which counts up from zero in minutes and seconds ("Time=MM:SS").

\* When the clock reaches three minutes, the following display appears:

Record Temperature Time=MM:ss Blk=40.0C

- 3.1.11 Measure the temperature of well E1 using the digital thermometer. Record this temperature as T(40).
- 3.1.12 Press ENTER.
  - \* The temperature of the sample block and heated cover will go to 95°C, and the following display will appear:

Going to 95°C... Cvr= xxC Blk = xx.xC

This display shows the current temperature of the block cover (Cvr=xxC) and sample block (Blk=xx.xC).

\* When the temperature of the block cover is within ten degrees of the sample block temperature, the following display appears:

Wait 3 minutes Time=MM:SS Blk=95.0C

This display shows the current sample block temperature ("Blk=95.0C") and a clock, which counts up from zero in minutes and seconds ("Time=MM:SS").

\* When the clock reaches three minutes, the following display appears:

Record Temperature Time=MM:SS Blk=95.0C

3.1.13 Measure the temperature of well E1 using the digital thermometer. Record this temperature as T(95).

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#### 3.2 **Test Results**

NOTE: Refer to Appendix I for the appropriate worksheet to record the results.

Perform the following steps to calculate the results of the test:

Use the following formula to calculate the average block temperature at 95°C hold: 3.2.1

Block Average at  $95^{\circ}C = T(95)$  - High Offset for the individual thermal cycler. (The High Offset value can be obtained from the label with the instrument's specifications provide with the specific 9600 thermal cycler).

- \* If the block average is more than 0.75°C above or below 95°C, your GeneAmp PCR System 9600 must be recalibrated.
- 3.2.2 Use the following formula to calculate the average block temperature at the 40°C hold.

Block Average at  $40^{\circ}$ C = T(40) - Low Offset for the individual thermal cycler. (The Low Offset value can be obtained from the label with the instrument's specifications provide with the specific 9600 thermal cycler).

\* If the block average is more than 0.75°C above or below 40°C, your GeneAmp PCR System 9600 must be recalibrated.

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4 DNA THERMAL CYCLER 9600 TEMPERATURE UNIFORMITY TEST

4.1 Testing the Temperature Uniformity

Perform the procedure as described in the following steps:

- 4.1.1 If the sample block heated cover is in the forward position, turn the cover knob completely counterclockwise, then slide the cover back.
- 4.1.2 Coat all the wells in sample block rows A, C, E, and H with mineral oil using a cotton swab.
- 4.1.3 Place the probe tray on the sample block with the notch facing the front of the instrument.
- 4.1.4 Place the probe assembly into wells A1 and A2 so that the dummy probe sits in A2. Carefully thread the probe wire through the notch in the probe tray.
- 4.1.5 Slide the heated cover forward and turn the cover knob clockwise until the white mark on the knob and the white mark on the cover are aligned.
- 4.1.6 Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.
- 4.1.7 Turn on the GeneAmp PCR System 9600 and create a two-temperature CYCLE program with the following parameters:

Setpoint #1 Temperature = 95°C Hold Time = 2:00 minutes Ramp Time = 0:00 minutes

Setpoint #2 Temperature =  $40^{\circ}$ C Hold Time = 2:00 minutes Ramp Time = 0:00 minutes

Cycles = 99

- 4.1.8 On the third cycle, measure the temperature of well A1 90 seconds into Setpoint #1 (95°C setpoint temperature) using the digital thermometer. The time remaining on the clock runtime display will read "0:30" (30 seconds). Record this temperature.
- 4.1.9 Still on the third cycle, measure the temperature of well A1 90 seconds into setpoint #2 (40°C setpoint temperature) using the digital thermometer. The time remaining on the clock runtime display will read "0:30" (30 seconds). Record this temperature.
- 4.1.10 After you measure the second temperature of well A1, turn the cover knob completely counterclockwise, then slide the heated cover back.
- 4.1.11 Move the probe assembly to wells A4 and A5, placing the dummy probe in A5.

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- 4.1.12 Slide the heated cover forward, then turn the cover knob clockwise until the white mark on the knob and the white mark on the cover are aligned. Repeat steps 4.1.8 and 4.1.9 for fourth cycle.
- 4.1.13 Repeat the measurements (as specified in 4.1.8 and 4.1.9 for cycles 4 and so on) on wells A4, A8, A12, C1, C4, C8, C12, E1, E4, E8, E12, H1, H4, H8, and H12. Make sure you place the measuring cone of the probe assembly into these wells and the dummy probe into adjacent wells.
- 4.1.14 After you have completed all measurements, remove the probe assembly from the sample block and turn off the digital thermometer.
- 4.1.15 Clean the oil from the sample block using cotton swabs.
- 4.2 Test Results

**NOTE**: Refer to Appendix I for the appropriate worksheet to record the results.

- 4.2.1 For the 16 Setpoint #1 measurements (95°C hold), subtract the lowest measured temperature from the highest measured temperature.
- 4.2.2 For the 16 Setpoint #2 measurements (40°C hold), subtract the lowest measured temperature from the highest measured temperature.
- \* If either result is more than 1°C, your GeneAmp PCR System 9600 must be serviced by a Perkin-Elmer Service Representative.

**♦END**